Differences in Temperature Dependence of Early Development of Sea Urchins with Different Growing Seasons

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Abstract. Three species of sea urchin, *Hemicentrotus pulcherrimus, Anthocidaris crassispina,* and *Hemicentrotus depressus,* inhabit the tidal areas around the Misaki Marine Biological Station, on the Pacific coast of Japan. These sea urchins have different breeding seasons and hence their embryos show differences in temperature sensitivity. In this study, differences in the temperature dependence of their embryogenesis were examined. It was found that the upper and lower limits of the optimal temperature range for embryogenesis in each animal were species-specifically determined, and that the range of seawater temperature during the breeding season was within this optimal temperature range. A quasi-linear relationship was obtained between temperature and relative developmental velocity, which was defined as the ratio against the maximal velocity attained at the highest temperature within the optimal temperature range. Based on this result, a form of graphical analysis was devised to represent the differences in species-specific developmental temperature sensitivity. The physiological and ecological significance of species-specific temperature sensitivity was also discussed.

Introduction

Several reports have discussed the temperature dependence of early development of sea urchins (Loeb and Wasterneys, 1911; Koehler, 1912; Loeb and Chamberlain, 1915; Hörstadius, 1925; Needham, 1931; Tyler, 1936; Hoadley and Brill, 1937; Bougis, 1971; Stephens, 1972a, b; McEdward, 1985; Bosch *et al.*, 1987), amphibi-

ans (Hertwig, 1898; Ryan, 1941; Moore, 1942; Volpe, 1953a, b; Bachmann, 1969) and other animals such as an insect (Krogh, 1914a), copepods (Herzig, 1983a), and rotifers (Herzig, 1983b). Relationships between the rate of development of embryos and temperature during the breeding season have been reported only for amphibians in North America (Ryan, 1941; Moore, 1942; Volpe, 1953a, b; Bachmann, 1969).

I observed similar relationships in three sea urchin species living in the same locality. In the tidal and subtidal areas around the Misaki Marine Biological Station, Kanagawa Prefecture, on the Pacific coast of Japan (139°6′ E, 35°2′ N.L.), *Hemicentrotus pulcherrimus* spawns in winter to early spring (from January to April) when seawater temperature is between about 10° and 15°C. *Anthocidaris crassispina* spawns in summer (from July to early September) when the temperature is between about 20° and 27°C. *Pseudocentrotus depressus* spawns in autumn to early winter (from October to December) when the temperature is between about 13° and 23°C. Each seawater temperature range in each spawning season is optimal for the embryos of the respective sea urchin species. However, the relationship between temperature and the developmental rates of these three species has never been investigated. This report presents an analysis of the effect of temperature on the developmental rates of these three species of sea urchin, and discusses the ecological significance of the results.

Materials and Methods

The sea urchins, *Hemicentrotus pulcherrimus, Anthocidaris crassispina,* and *Pseudocentrotus depressus* were

collected from the tidal area around the Misaki Marine Biological Station, Kanagawa Prefecture, on the Pacific coast of Japan (139°6′ E, 35°2′ N.L.). Experiments were always conducted in the peak spawning season for these sea urchins.

Eggs and sperm were obtained by introducing 0.5 M KCl into the body cavity. The eggs used were always obtained from only one mature individual to avoid mixing with eggs from premature or overmature individuals and to ensure optimal synchrony. The eggs were divided into groups and each group was inseminated after preincubation at a specific temperature, followed by culture with gentle stirring using a glass blade. To optimize developmental synchrony, all cultures were maintained in constant temperature water bathes adjusted with an accuracy of ±0.1°C as read with a standard thermometer.

Optimal temperature was defined as the temperature at which the developmental process from early cleavage to pluteus larva was able to proceed without any abnormal morphology. The upper and lower limits of the optimal temperature range for the development of each sea urchin species was experimentally determined by culturing embryos at temperatures increasing and decreasing at intervals of 1°C.

The times of the first, second, third, and fourth cleavage, hatching, mesenchyme ingression, and gastrulation, defined as the number of minutes after insemination when about 50% of the embryos had completed each stage, were measured. The size of the counted sample population was 50 to 60 embryos and the counting interval was 5 min for the first, second, third, and fourth cleavage, or 15 min for the stages later than hatching.

The reciprocal of the time required for embryos to develop into each stage was taken as the developmental velocity. The velocities were temperature-dependent, and maximal at the highest temperature within the optimal range temperature for each species. To clarify species-specific differences in the temperature dependence of developmental velocity, I introduced the relative developmental velocity defined as the ratio $(1/t_T)/(1/t_H)$, where t_T is the time required for embryos to develop into each stage at a specific temperature and t_H is the time required to develop into the respective stage at the highest temperature within the optimal temperature range.

Results

Hemicentrotus pulcherrimus embryos showed normal development within the temperature range of 5°C to 23°C. Normal development was shown by *Pseudocentrotus depressus* embryos within a range of 9°C to 25°C. Those of *Anthocidaris crassispina* showed normal development within a range of 16°C to 29°C. At 4°C, cleavage

Table I

Range of optimal temperature for early development of three species of sea urchin, Hemicentrotus pulcherrimus, Pseudocentrotus depressus, *and* Anthocidaris crassispina, *and range of seawater temperature during their breeding seasons around tidal areas at the Misaki Marine Biological Station on the Pacific coast of Japan*

Species	Range of optimal temperature for early development (°C)	Range of seawater temperature during breeding season (°C)
Hemicentrotus pulcherrimus	5–23	10–17
Pseudocentrotus depressus	9–25	14–21
Anthocidaris crassispina	16–29	19–25

Optimal temperature for early development was defined as the temperature at which sea urchin embryos were able to develop into pluteus larvae without any abnormal morphology. Optimal temperature ranges were determined experimentally. Seawater temperature was measured at a depth of 1 m at a point in the tidal area around the Misaki Marine Biological Station. The temperature was taken once every week. The range of seawater temperature during the breeding season indicates the maximum fluctuation of the temperature, *i.e.*, the range between the highest and the lowest temperature during the season.

of *H. pulcherrimus* embryos was abnormal; the embryos were unable to form blastulae. The embryos showed no developmental change other than elevation of the fertilization membrane at temperatures lower than 3°C. At 15°C, *A. crassispina* embryos were unable to form normal plutei, at 14°C they were unable to develop into gastrulae, and at temperatures lower than 13°C they showed no blastula formation. The development of the embryos was nearly arrested at temperatures lower than about 10°C. At temperatures higher than the ranges optimal for normal development, the embryos of any species were unable to develop normally to the gastrula stage. Since the ranges of seawater temperatures during the spawning season are about 10°C to 15°C for *H. pulcherrimus*, about 13°C to 23°C for *P. depressus*, and about 20°C to 27°C for *A. crassispina* (Table I), it is apparent that the ranges of temperature optimal for normal embryogenesis of each species of sea urchin sufficiently cover those of seawater during the spawning season of each respective animal.

Within the ranges of temperature optimal for normal development of the sea urchin embryos, temperature had a significant effect on the velocity of development. Table II shows the times necessary for development of *H. pulcherrimus* into the first, second, third, and fourth cleavage stages, hatching, mesenchyme ingression, and

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Table II

Effect of temperature on the times or the relative developmental velocity for Hemicentrotus pulcherrimus *embryos to arrive at each developmental stage*

	Temperature (°C)					
	5	10	12	15	2	23
Stage						
2-Cell	343 ± 6	162 ± 6	127 ± 2	90 ± 0	65 ± 0	60 ± 0
	(0.18 ± 0.01)	(0.37 ± 0.02)	(0.47 ± 0.01)	(0.67 ± 0.00)	(0.92 ± 0.00)	
4-Cell	513 ± 12	245 ± 15	188 ± 8	137 ± 2	97 ± 2	90 ± 0
	(0.18 ± 0.01)	(0.38 ± 0.01)	(0.49 ± 0.01)	(0.66 ± 0.01)	(0.93 ± 0.02)	
8-Cell	703 ± 12	323 ± 20	263 ± 26	192 ± 13	133 ± 5	122 ± 2
	(0.17 ± 0.01)	(0.38 ± 0.02)	(0.48 ± 0.01)	(0.66 ± 0.01)	(0.92 ± 0.00)	
16-Cell	888 ± 43	412 ± 28	330 ± 29	240 ± 10	170 ± 10	155 ± 5
	(0.18 ± 0.01)	(0.38 ± 0.01)	(0.48 ± 0.02)	(0.65 ± 0.01)	(0.93 ± 0.01)	
Hatching	2740 ± 348	1447 ± 105	1127 ± 88	865 ± 53	633 ± 21	620 ± 22
	(0.21 ± 0.01)	(0.43 ± 0.02)	(0.55 ± 0.02)	(0.72 ± 0.02)	(0.98 ± 0.00)	
Mesenchyme ingression	4150 ± 40	1800 ± 30	1440 ± 30	1010 ± 10	735 ± 5	665 ± 5
	(0.16 ± 0.00)	(0.37 ± 0.00)	(0.47 ± 0.01)	(0.66 ± 0.00)	(0.91 ± 0.00)	
Gastrulation	5035 ± 35	2170 ± 20	1755 ± 15	1250 ± 10	890 ± 10	815 ± 5
	(0.16 ± 0.00)	(0.38 ± 0.00)	(0.47 ± 0.01)	(0.65 ± 0.00)	(0.92 ± 0.01)	

Temperatures were adjusted to an accuracy of ±0.1°C. Numbers indicate times (minutes) taken for 50% of the embryos to arrive at each stage. Numbers in parentheses are relative velocities, the velocity at a given temperature being defined as the ratio of the reciprocal of the time required for development into each stage to the reciprocal of the time required to develop into the corresponding stage at the highest temperature. Each number is the average ± standard error for separate three experiments.

gastrulation at various temperatures within the ranges of temperature optimal for the embryos of this species. As it was easier to measure the times required for development into the first, second, and third cleavage stages and hatching more accurately than those of mesenchyme ingression, gastrulation, and subsequent stages, Table III lists only the times required for development into the first, second, and third cleavages and hatching for the other two species of sea urchin. The values in parentheses in Tables II and III indicate relative developmental velocities. It is obvious that at a specific temperature the relative velocities of all stages except hatching are nearly equal, and hence it can be said that the relative velocity is dependent only on temperature, and not on the developmental stage. Figure 1 presents the results shown in Table II in graph form, and it is apparent that a quasi-linear relationship exists between temperature and the relative velocity of every stage within each range of optimal temperature. In addition to this, the plots in Figure 1 overlap into a single quasi-linear curve, indicating that the relative velocities from fertilization to every stage except hatching can be plotted on such a curve. Extrapolation of the curve gives a temperature (3°C) at which the velocity of development is zero (T_0C).

It is noted that the quasi-linear curve designating the relationship between the relative velocity of hatching and temperature deviated from those of the other stages and temperatures. This deviation was observed in the embryos of the other species.

The relative velocities of development to the first, second, and third cleavages were then plotted on single curves for each of the three species of sea urchin, and the curves were found to be individually characteristic (Fig. 2). The data on which these curves were based are shown in Tables II and III. The differences between the curves indicate the differences in the relationships between temperature and velocity in the embryos of *H. pulcherrimus, P. depressus,* and *A. crassispina.* These differences clearly reflect the differences in the optimal range of temperature for embryos of each species of sea urchin.

The differences in temperature dependence of their early development are thus indicated by the differences in the positions of the curves in the coordinates of temperature and relative developmental velocity. It should also be noted that within the range of seawater temperatures during the spawning season, the range of relative developmental velocity was nearly constant (from about 0.4 to 0.8), regardless of the species of sea urchin.

Discussion

Temperature is a primary limiting factors affecting a wide range of biological phenomena—from metabolic biochemical reactions to the geographical distributions

Table III

Effect of temperature on the times or the relative developmental velocity for Anthocidaris crassispina *and* Pseudocentrotus depressus *embryos to arrive at each developmental stage*

Anthocidaris crassispina	Temperature (°C)				
	16	20	23	26	29
Stage					
2-Cell	178 ± 3	105 ± 5	65 ± 0	50 ± 0	40 ± 0
	(0.23 ± 0.01)	(0.38 ± 0.02)	(0.62 ± 0.00)	(0.80 ± 0.00)	
4-Cell	293 ± 7	168 ± 7	105 ± 5	80 ± 5	60 ± 0
	(0.20 ± 0.01)	(0.38 ± 0.01)	(0.63 ± 0.02)	(0.81 ± 0.01)	
8-Cell	405 ± 15	230 ± 10	145 ± 5	113 ± 3	93 ± 2
	(0.23 ± 0.01)	(0.41 ± 0.01)	(0.64 ± 0.01)	(0.83 ± 0.01)	
16-Cell	520 ± 20	290 ± 10	183 ± 2	137 ± 3	113 ± 2
	(0.22 ± 0.01)	(0.39 ± 0.01)	(0.61 ± 0.01)	(0.82 ± 0.01)	
Hatching	1130 ± 100	715 ± 65	513 ± 38	420 ± 30	355 ± 25
	(0.31 ± 0.01)	(0.50 ± 0.01)	(0.69 ± 0.01)	(0.85 ± 0.01)	

Pseudocentrotus depressus	Temperature (°C)				
	10	15.5	20	23	25
Stage					
2-Cell	265 ± 5	128 ± 3	88 ± 3	75 ± 0	70 ± 0
	(0.27 ± 0.01)	(0.56 ± 0.01)	(0.79 ± 0.01)	(0.93 ± 0.00)	
4-Cell	385 ± 5	195 ± 5	128 ± 3	108 ± 3	103 ± 3
	(0.27 ± 0.01)	(0.53 ± 0.00)	(0.81 ± 0.01)	(0.95 ± 0.00)	
8-Cell	500 ± 20	255 ± 5	173 ± 3	143 ± 3	138 ± 3
	(0.28 ± 0.01)	(0.54 ± 0.01)	(0.80 ± 0.01)	(0.93 ± 0.00)	
16-Cell	610 ± 30	318 ± 8	215 ± 5	178 ± 3	168 ± 3
	(0.28 ± 0.01)	(0.53 ± 0.01)	(0.78 ± 0.01)	(0.94 ± 0.00)	
Hatching	1770 ± 30	975 ± 15	655 ± 5	538 ± 8	455 ± 5
	(0.26 ± 0.01)	(0.47 ± 0.01)	(0.70 ± 0.01)	(0.85 ± 0.01)	

Legend is same as for Table II. Each number is the average ± standard error for two separate experiments.

of species. Of course, development is also dependent on temperature. Some early embryologists tried to determine whether the Arrhenius law could be applied to developmental velocity. There was even a report claiming that the law indeed held for the relationship between temperature and developmental velocity (Crozier, 1926), but contrary to this contention, it has since become evident that the law can hardly be applied to this relationship. Instead, it has been proved that the relationship can be represented as a sigmoidal curve, the major part of which is linear (Loeb and Wasterneys, 1911; Krogh, 1914b; Loeb and Chamberlain, 1915; Atlas, 1935; Tyler, 1936; Hoadley and Brill, 1937; Ryan, 1941; Volpe, 1953a, b; Bachmann, 1969; Stephens, 1972a). This is also confirmed in the present work. Despite attempts by several authors to analyze these relationships graphically (Krogh, 1914a, b; Crozier, 1926; Bělehrádek, 1930; Atlas, 1935; Tyler, 1936; Bachmann, 1969), no clear, sharp, and precise analysis has yet been achieved.

In addition, except for the stage of hatching, the curves representing the relationship were confirmed to be nearly the same in the present experiment, regardless of the developmental stages of the sea urchins used. This phenomenon was first noticed by Atlas in the embryogenesis of *Rana pipiens* (Atlas, 1935), and was also found to be true when I re-examined previously published data on the early development of *Arbacia punctulata* (Loeb and Wasterneys, 1911; Loeb and Chamberlain, 1915; Hoadley and Brill, 1937), *Strongylocentrotus droebachiensis* (Stephens, 1972a), and *Dendraster excentricus* (Tyler, 1936). Hence, this phenomenon seems to be universally valid for early development of animals of any kind, within the range of optimal temperature. Thus it can be regarded as an embryological law.

It is noteworthy that the curve indicating the relationship between the relative velocity of hatching and temperature deviated. The reason for this deviation is unknown, but it should be pointed out here that, in comparison with cleavage and morphogenetic movement, hatching is a relatively simple biochemical process, *i.e.*,
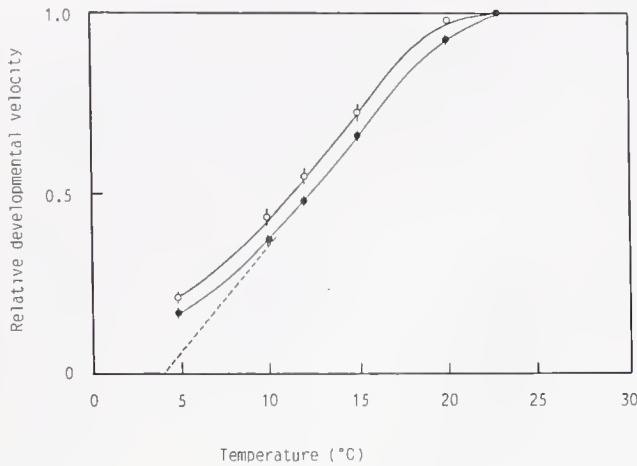
Figure 1. Relationship between temperature and the relative developmental velocity of *Hemicentrotus pulcherrimus* embryos.

The results are based on the data shown in Table II. Each solid circle and bar represents the mean and standard error respectively, of the relative velocities for development into the first, second, third, and fourth cleavages, mesenchyme ingression, and gastrula stage. Each open circle and bar indicates respectively the mean and standard error of the relative velocity for development into the hatching stage at a given temperature. The dotted line is an extrapolation of the curve, assuming it to be a straight line.

exocytosis of hatching enzyme and its hydrolytic action on the fertilization membrane.

The factors determining the temperature tolerance or dependence of embryos have not yet been elucidated. Bělehrádek (1930) proposed that in general biological processes including development protoplasmic viscosity is the main factor determining the effect of temperature. Stephens (1972b) has tried to explain the temperature tolerance of embryos in terms of Inoué's mitotic dynamic theory. Cellular activities in early embryogenesis, such as cleavage and cell adhesion (Fujisawa and Amemiya, 1988) are significantly affected by temperature, and these processes are mainly dependent upon cytoskeletal activities. However, cytoskeletal elements do not seem to be a candidate for a factor, since actin, as well as tubulin, is reported to be evolutionarily stable (Loewy, 1952; Hatano and Oosawa, 1966; Adelman and Taylor, 1969; Luduena and Woodward, 1973; Krauhs *et al.,* 1981; Hall *et al.,* 1983; Sullivan and Cleveland, 1984). Temperature sensitivities of enzymes of DNA synthesis may also be another important factor since mitosis is closely connected with DNA synthesis. It is noteworthy that the temperature optimum of DNA polymerase in *Strongyrocentrotus purpuratus* and *Lytechinus pictus* embryos is 30°C (Loeb, 1969) as well as of DNA topoisomerase (Poccia *et al.,* 1978).

Control of the timing mechanism of gametogenesis in

response to temperature is another important problem that would be useful to elucidate, since this mechanism is directly related to embryogenesis and hence affects the proliferation of animals as well as the distribution of mature adults. There have been several reports on optimal temperature response of gametogenesis (Barnes, 1963; Fries, 1964; Meats and Khoo, 1976; Shrode and Gerking, 1977). However, this problem is beyond the scope of the present work.

The ecological significance of the temperature dependence of development has been investigated only in amphibians of North America (Ryan, 1941; Moore, 1942; Volpe, 1953a, b; Bachmann, 1969), but the present results confirm the ecological significance of temperature dependence of this phenomenon. Fortunately, three types of ecological elements, *H. pulcherrimus,* a coldtemperate element, *P. depressus,* a medium-temperate element, and *A. crassispina,* warm-temperate elements, were found to coexist in the tidal and subtidal area around the coast near the Misaki Marine Laboratory. This area is the southern limit for *H. pulcherrimus* while it is the northern limit for *A. crassispina,* and thus it seems reasonable that the embryos of *H. pulcherrimus* should develop in winter while those of *A. crassispina* develop in summer. Temperature tolerance seems to be narrower and stricter in the embryos than in the adults of these species, and this tolerance characteristic is thought to determine the distributions of the animals.
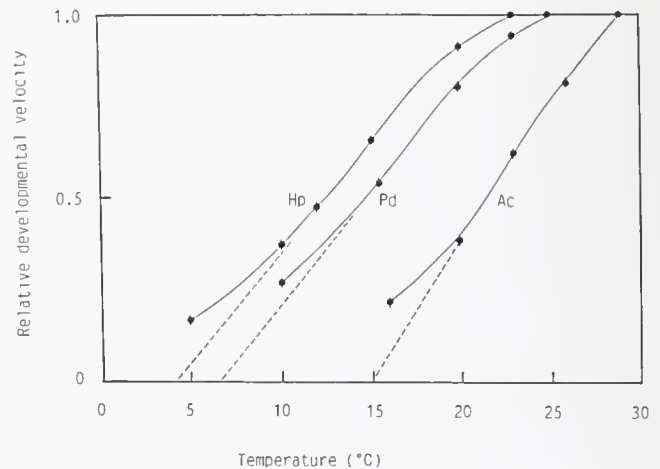

Figure 2. Relationship between temperature and the relative developmental velocity of the three species of sea urchin, *Hemicentrotus pulcherrimus, Anthocidaris crassispina,* and *Pseudocentrotus depressus.*

The results are based on the data in Tables II and III. Each value represents the average ± standard error of the relative velocities for development into the first, second, third and fourth cleavage stages at a given temperature. Hp, *Hemicentrotus pulcherrimus;* Pd, *Pseudocentrotus depressus;* Ac, *Anthocidaris crassispina.*

Diadema setosum may be another example. This sea urchin lives widely in the Indo-Pacific sea and spawns throughout the year in the tropics where seawater temperatures are nearly always above 25°C (Mortensen, 1937, 1938; Pearse, 1974). In Sagami Bay and neighboring Suruga Bay on the south coast of Japan, the northern limit of the distribution of this species, the animal spawns once a year, *i.e.*, on the days of full moon in August (Yoshida, 1952). I am now attempting to clarify whether the temperature tolerance hypothesis holds for this species of sea urchin. Similar examples may also be found in *Arbacia punctulata* and *Strongyrocentrotus droebachiensis*, which live in Cape Cod Bay. Based on data from Loeb and Wasterneys (1911), Loeb and Chamberlain (1915), Hoadley and Brill (1937), and Stephens (1972a), clear correlations seem to be established between the spawning seasons of these species, seawater temperature, and the temperature tolerance of their embryos. Various species of sea urchin have a wide range of distribution: from equatorial to arctic and antarctic seas, and from shallow to deep seas. It is hoped that investigators will soon confirm whether the hypothesis holds globally for these sea urchins.

Acknowledgments

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